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ORIGINAL ARTICLE

Comparative Optimization of HPLC Conditions for The Isolation of Caftaric Acid from *Ailanthus excelsa* using Hydroalcoholic Extraction

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ABSTRACT
This study describes the development and optimization of a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the isolation of caftaric acid from the hydroalcoholic extract of Ailanthus excelsa. Dried plant material was subjected to Soxhlet extraction using ethanol–water (70:30 v/v), and the crude extract was analyzed and characterized. The chromatographic separation was carried out on an Agilent C18 column under different methanol–phosphate buffer compositions and detection wavelengths. The optimization focused on achieving sharp peaks, appropriate retention times, and baseline stability. The optimized conditions—mobile phase (methanol:phosphate buffer, 55:45 v/v, pH 2.5), column temperature (30°C), detection at 320 nm, and flow rate of 1.0 mL/min—provided rapid and selective separation of caftaric acid at 3.37 minutes. This method offers a robust and efficient protocol for analyzing caftaric acid and supports its use as a green analytical tool in quality control of A. excelsa extracts.
Keywords: *Ailanthus excelsa, caftaric acid, HPLC optimization, hydroalcoholic extraction, green analytical chemistry, reversed-phase chromatography*

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INTRODUCTION

Ailanthus excelsa Roxb. (family Simaroubaceae) is a well-regarded medicinal tree distributed throughout India and widely utilized in traditional medicine systems. Various parts of the plant, including its bark and leaves, are known for their rich phytochemical composition, including phenolics, flavonoids, glycosides, and organic acids that contribute to its diverse pharmacological activities. The plant has been investigated for its anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective properties, making it an important candidate for the development of safe and efficacious herbal formulations [1–4].

Among the many phytoconstituents present in *A. excelsa*, caftaric acid—a hydroxycinnamoyl tartaric acid derivative—stands out as a prominent phenolic compound. Caftaric acid is of particular interest due to its broad-spectrum biological activities, including antioxidant, anti-inflammatory, and cytoprotective effects (5–7). Its role in enhancing cellular defense mechanisms against oxidative stress has prompted its exploration as a bioactive marker compound for the quality control of *A. excelsa*-based preparations [8–10].

To obtain bioactive metabolites like caftaric acid efficiently and sustainably, green extraction methods utilizing hydroalcoholic solvent systems have gained increasing preference. Hydroalcoholic solvents (e.g. ethanol–water mixtures) allow selective extraction of target phytochemicals with minimal environmental impact [11,12]. This also aligns with green chemistry principles, supporting the development of environmentally responsible research protocols.

Despite its significance, accurate quantification and isolation of caftaric acid from *A. excelsa* require a robust, optimized chromatographic method [13]. High-performance liquid chromatography (HPLC) is the most accepted and powerful analytical technique for separating, detecting, and quantifying phenolic compounds due to its sensitivity, reproducibility, and scalability [14–16]. However, the retention behavior, peak resolution, and signal intensity of caftaric acid can vary significantly depending on

parameters such as column chemistry, mobile phase composition, pH, temperature, and detection wavelength.

In this context, the present study aims to optimize a reversed-phase HPLC method to isolate caftaric acid from the hydroalcoholic extract of ***A. excelsa***. By systematically varying experimental parameters and monitoring their impact on retention time, peak shape, baseline stability, and sensitivity, we establish a rapid, green, and reproducible analytical protocol. The results serve as a stepping stone for future method validation and can support the standardization of herbal products containing ***A. excelsa*** and its active constituents.

MATERIAL AND METHODS

Collection of Plant Material and Authentication

Ailanthus excelsa herb was collected in the month of January 2024 from local area of Buldhana, Maharashtra, India 443001. From the collected plant material, the herbarium was prepared individually and authenticated by Department of Botany Shri Shivaji Science and Art College, Chikhli, Buldhana. Reference number CS/Certificate/735 was deposited.

Soxhlet Extraction using Hydroalcoholic Solvent

Ailanthus excelsa leaves and barks were collected and washed them using distilled water to remove any dust or foreign particle, and then air-dried in the shade for a week at room temperature. It is essential to dry it in room temperature to avoid the loss of volatile phytoconstituents. About 500 grams of dried plant material was grinded into a fine powder for further extraction. The resulting powder was subjected for Soxhlet extraction using Soxhlet apparatus by hydroalcoholic solvent (Ethanol: water; 70:30). At least 48 hours were devoted to the extraction process, during which time several syphon cycles were completed. The dark green hue of the solvent indicated that the greatest amount of phytoconstituents had been extracted from the sample. The solvent was then evaporated off the obtained extract at room temperature. The crude extract obtained from this process was further utilized for subsequent investigation [17–19]. The obtained extract was subjected for phytochemical screening and quantitative analysis. The deep green color of the solvent indicates excellent extraction of the phytoconstituents. After extraction, the solvent was meticulously evaporated at ambient temperature to obtain a crude extract. We further analyzed the crude extract by phytochemical screening and quantitative assessment to identify and quantify its distinct ingredients. This analytical technique allows for a comprehensive examination of the chemical composition of the plant, potentially uncovering molecules with notable pharmacological attributes [17–19].

Compound Isolation of Caftaric acid from *Ailanthus excelsa*

Instrumentation

The chromatographic analysis of Caftaric acid was carried out using a high-performance liquid chromatography (HPLC) system equipped with a quaternary pump, autosampler, column oven, and a photodiode array (PDA) detector set to 320 nm for detection. The separation was performed using an Agilent C18 column (150 mm × 4.6 mm, 5 µm), which was maintained at a constant temperature of 30°C. Data acquisition and processing were managed through Empower 3 software.

Preparation of Mobile Phase

The mobile phase consisted of a mixture of methanol and phosphate buffer in the ratio of 55:45 (v/v). The phosphate buffer was prepared by dissolving 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) in 1000 mL of HPLC-grade water. The pH was adjusted to 2.5 using dilute orthophosphoric acid. The buffer solution was filtered through a 0.45 µm membrane filter and degassed using ultrasonication. After preparation, methanol was mixed with the buffer in the prescribed ratio, and the final mobile phase was again filtered and degassed to remove any dissolved gases [20–22].

Preparation of Standard Solution of Caftaric Acid

An accurately weighed amount of 10 mg of Caftaric acid reference standard was transferred into a 10 mL volumetric flask. It was dissolved in methanol, and the volume was made up to the mark with methanol to yield a stock solution of 1000 µg/mL [23,24].

Preparation of Sample Solution

A suitable quantity of the sample, equivalent to approximately 10 mg of Caftaric acid, was accurately weighed and transferred to a 100 mL volumetric flask. About 70 mL of methanol was added, and the mixture was sonicated for 15–20 minutes to ensure complete extraction. The solution was then diluted to volume with methanol and mixed thoroughly. The resulting solution was filtered through a 0.45 µm membrane filter, and the clear filtrate was collected in an HPLC vial for injection into the chromatographic system.

Chromatographic condition

The analysis was performed using an Agilent C18 column (150 mm × 4.6 mm, 5 µm) maintained at 30°C. The mobile phase consisted of methanol and phosphate buffer (55:45 v/v) with pH adjusted to 2.5. Detection was carried out at 320 nm using a photodiode array detector. The flow rate and injection volume should be specified to complete the chromatographic conditions.

Method development

The method development for the analysis of Caftaric acid involved optimizing several parameters. The selection of the Agilent C18 column provided suitable retention and separation of the analyte. The mobile phase composition of methanol and phosphate buffer (55:45 v/v) was determined to achieve optimal peak resolution and shape. The pH of the buffer was adjusted to 2.5 to ensure proper ionization of the analyte. The column temperature was set at 30°C to maintain consistent retention times. Detection wavelength was set at 320 nm based on the absorption maximum of Caftaric acid. Standard and sample preparation procedures were established to ensure accurate quantification. The method should be further validated for parameters such as linearity, precision, accuracy, and specificity to ensure its reliability for the intended application.

RESULTS AND DISCUSSION

Organoleptic and Physicochemical Analysis of Extract

Ailanthus excelsa (AE) extract were subjected for organoleptic properties analysis. The organoleptic properties of the obtained extract are tabulated in Table 1. The results of physicochemical analysis are exemplified in Table 2.

Table 1: The organoleptic properties of extracts

Parameters	AE
Color	Greenish yellow
Odor	Aggregable and unpleasant
Taste	Bitter
Texture	Resinous
% yield	9.4

Table 2: The physicochemical analysis of extracts

Parameters	AE
pH	
1% Solution	7.3
10% Solution	8.9
Foreign content	0%
LOD	3.45%
Ash values	
Total Ash value	18.44%
Acid insoluble ash value	3.12%
Water soluble ash value	5.72%
Sulphated ash value	2.91%
Extractive values	
Alcohol-soluble extractive	1.04%
Water-soluble extractive	1.98%
Heavy metals estimation (present)	Absent
Pesticide residues	Absent

Organoleptic testing includes judging how a thing tastes, smells, looks, and feels in the mouth. Products must undergo organoleptic testing to ensure they are up to par with what the company and the customer have agreed upon. Assessing the organoleptic qualities of a product is crucial to establishing its commercial viability and storage life. It is important to look at the sensory experience of a product even if research shows that it is safe and meets nutritional promises. All of the senses (taste, smell, touch, sight, and hearing) are involved. The extract met the criteria for acceptable organoleptic qualities. Physicochemical analysis was used to investigate the following chemical properties of the test substances, all of which have been recognised as major structural components contributing to penetration, irritation, or sensitization: In order to establish whether medicines have been tampered with or contaminated, the physicochemical analysis of plant drugs is required. Determining the total ash content of different drugs is crucial for establishing their efficacy and purity. The term "foreign organic matter" is used to describe any material that has any of the aforementioned components. In addition to the components stated in the

definition and description, or the components for which the limit is provided in the specific monograph, other components of the organ or organs from which the medication is created may also be present.

Preliminary Phytochemical Screening of Extract

Phytochemical screening refers to the scientific method of analysing, studying, extracting, and testing with distinct classes of phytoconstituents contained in different areas of the base in order to find new medications. It would then be possible to remove the base's active ingredients for further study. Finding the bioactive components found in medicinal plants is a crucial step towards the discovery and development of novel medications, and the first step in this process is the screening of phytochemicals. The results of preliminary phytochemical screening are tabulated in Table 3. The presence of different phytochemicals in the extract indicates its significant role in different diseases.

Table 3: The results of preliminary phytochemical screening of extract

Chemical Test	Observations
	TP
Test for Carbohydrate	++
Test for reducing sugars	++
Test for monosaccharides	++
Test for Proteins	---
Test for amino acids	---
Test for Fats and Oil	+
Test for Steroids	---
Test for Cardiac Glycosides	--
Test for Anthraquinone Glycosides	+
Test for Saponin Glycoside	++
Test for Alkaloids	+++
Test for Tannins and Phenolic compounds	++
Test for Flavonoids	++

HPLC Method for isolation of Caftaric acid from *Ailanthus excelsa*

This study focused on optimizing the chromatographic conditions for isolating Caftaric acid from *Ailanthus excelsa*. Three trials were conducted, each with varying parameters, to improve the separation and detection of Caftaric acid. In the first trial (**Figure 1**), the initial chromatographic conditions utilized an Agilent C18 column (150 mm x 4.6 mm, 5 µm) with a mobile phase composition of methanol and phosphate buffer (pH 2.5) at a ratio of 70:30. The flow rate was set at 1.0 mL/min, with PDA detection in the range of 200-400 nm. The column temperature was maintained at 30°C, and the injection volume was 10 µL. Despite a run time of 10 minutes, the peak exhibited a longer retention time than desired. This extended retention time could potentially lead to broader peaks and reduced efficiency, necessitating further optimization of the method.

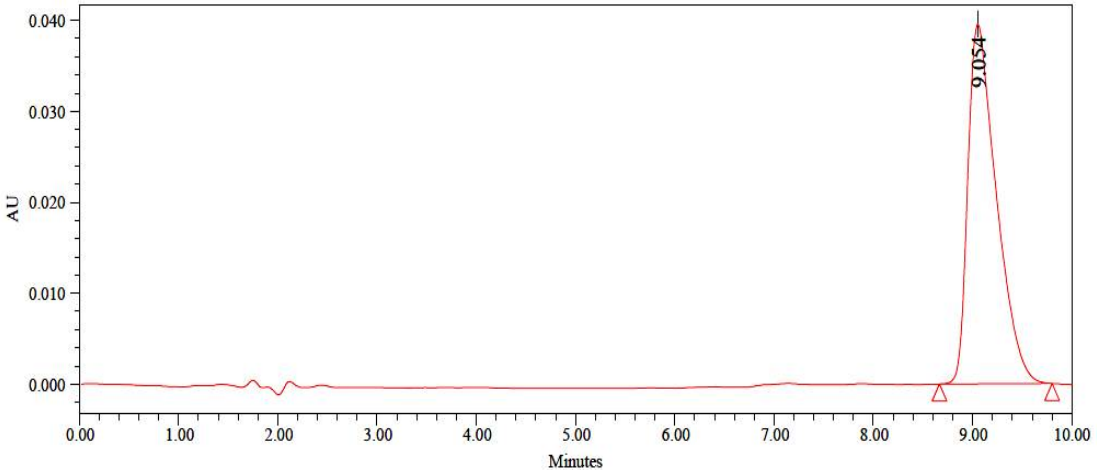

Figure 1: First Trial for Isolation of Caftaric acid

In second trial (**Figure 2**), the mobile phase composition was adjusted to methanol and phosphate buffer (pH 2.5) at a ratio of 60:40. The detection wavelength was specifically set to 310 nm, and the run time was extended to 20 minutes. The flow rate remained at 1.0 mL/min, column temperature at 30°C, and injection volume at 10 µL. However, the resulting chromatogram exhibited poor baseline stability, which could compromise the accuracy of peak integration and quantification.

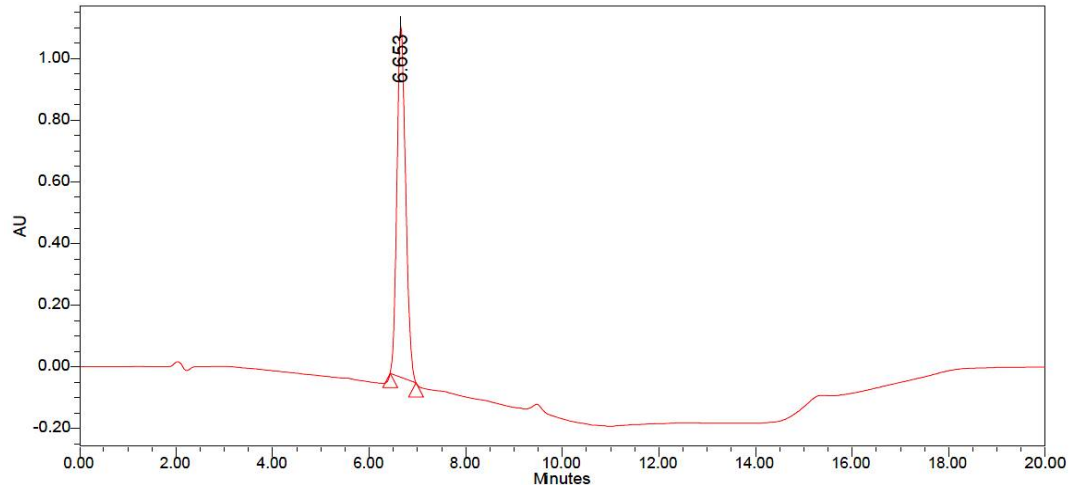

Figure 2: Second Trial for Isolation of Caftaric acid

The final optimized method for Caftaric Acid analysis employed the same Agilent C18 column with further adjustments. The mobile phase ratio was fine-tuned to 55:45 (methanol: phosphate buffer, pH 2.5) (**Figure 3**). The detection wavelength was optimized to 320 nm, corresponding to the maximum absorbance of Caftaric Acid. The run time was significantly reduced to 5 minutes, with the target analyte eluting at 3.368 minutes. The flow rate remained at 1.0 mL/min, column temperature at 30°C, and injection volume at 10 µL. This optimization resulted in improved peak shape, reduced analysis time, and enhanced overall chromatographic performance.

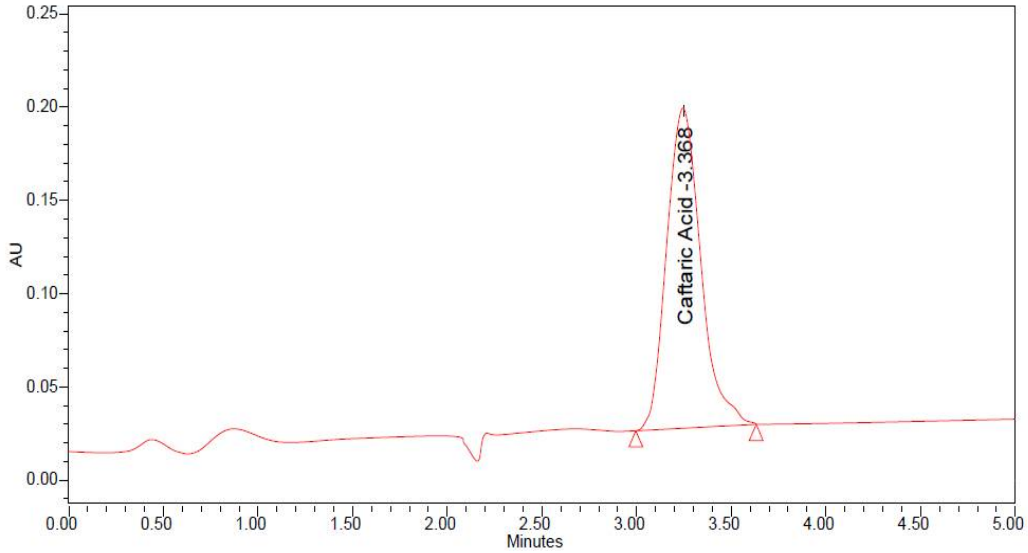

Figure 3: Third Trial for Isolation of Caftaric acid

The progression through these trials demonstrates the systematic approach to method development, addressing key parameters such as mobile phase composition, detection wavelength, and run time. The final method offers a rapid and efficient analysis of Caftaric Acid, suitable for routine quality control and research applications. Future work could focus on method validation, including assessments of linearity, precision, accuracy, and robustness to ensure the reliability of the method.

CONCLUSION

The present work successfully optimized a reversed-phase HPLC method for the selective isolation of caftaric acid from the hydroalcoholic extract of *Ailanthus excelsa*. Progressive trials revealed that using a methanol–phosphate buffer system at 55:45 (v/v), pH 2.5, with PDA detection at 320 nm yielded sharp, symmetrical peaks and a short retention time of 3.37 minutes. This optimized green-chemistry-driven HPLC method can serve as a rapid, reproducible tool for the routine quantification of caftaric acid in herbal extracts. Future validation studies focusing on linearity, precision, accuracy, and robustness will establish its broad utility for standardization of this medicinal plant extract.

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